Mycorrhizae : physiology and genetics - Les mycorhizes : physiologie et génétique.
I^re ESM/I^re SEM, Dijon, 1-5 July 1985.- INRA, Paris, 1986

Effects of different soils on the establishment and influence of sheathing mycorrhizas

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INTRODUCTION

Like others, forest ecosystems are dynamic, constantly changing as they respond to, and influence, their environments. It is therefore not surprising that the communities of fungi associated with forests also change (FRANKLAND, 1981). It is already well established that the non-parasitic microbes colonising the surfaces of ageing beech roots (rhizoplane microbes) change in an ordered sequence (HARLEY and WAID, 1955). LAMB (1979) more recently found that the types of fungi forming sheathing mycorrhizas became more diverse in association with ageing saplings of *Pinus elliottii* Little & Dorman. What is the nature of the changes recorded by Lamb? From observations made of the spatial and temporal occurrence of fruitbodies of sheathing mycorrhizal fungi in an ageing stand of *Betula* spp. MASON *et al.*, (1982) enunciated the concept of mycorrhizal succession in which species of *Hebeloma, Inocybe* and *Laccaria* are characteristically associated with young trees whereas those of *Amanita, Cortinarius* and *Russula* seem to be restricted to older trees. Evidence of similar temporal sequences has been reviewed by LAST and FLEMING (1984), including those associated with *Pinus radiata* D. Don and *Pseudotsuga menziesii* (Mirb.) Franco (Chu-Chou, 1979; CHU-CHOU and GRACE, 1981; 1983).

DEACON *et al.* (1983) established that a broad distinction can be made between early- and late-stage fungi (i.e. those occurring early and late in the mycorrhizal succession) based on differences in behaviour in unsterile soil. Early-stage fungi readily infect seedlings from resident or introduced inocula in unsterile soil whereas late-stage fungi cannot (DEACON *et al.*, 1983; DANIELSON *et al.*, 1984; LAST *et al.*, 1985). This distinction has made it possible to adopt a rational approach to the selection of fungi for inoculating tree seedlings (MASON *et al.*, 1983). However, more recent evidence, given in this paper, has indicated that the subsequent persistence of mycorrhizal associations is influenced by soil conditions so making it necessary to consider a tripartite system, host x fungus x soil.

RESULTS AND DISCUSSION

A. SOIL EFFECTS

When axenically germinated seedlings of silver birch, *Betula pendula* Roth. were subsequently grown in unsterile soil for 8 weeks in glasshouse conditions, it

was found that the origin of the soil profoundly influenced the types of mycorrhizas that developed thereafter (FLEMING *et al.*, 1984). When seedlings were grown in soil ('mycorrhizal-soil') it was found that most (68%) of the mycorrhizas that developed were attributable to *Inocybe*. In contrast, only a few mycorrhizas developed on seedlings grown in soil taken from beyond the edge of the stand ('non-mycorrhizal' soil) and these were attributable to *Hebeloma* sp.

All of the seedlings were subsequently transplanted to the 'non-mycorrhizal' soil and grown in glasshouse conditions for two growing seasons when the initial differences in mycorrhizal populations were still evident. The majority (77%) of seedlings propagated in 'mycorrhizal' soil still possessed mycorrhizas attributable to *Inocybe* although 72% of the total now also possessed 'brown with cream tip' mycorrhizas (BCT). Of the seedlings propagated in the 'non-mycorrhizal' soil, 94% had *Hebeloma* mycorrhizas, while 38% also had mycorrhizas formed with *Lactarius pubescens* (Fr.:Krombh.) Fr.

At the start of the third growing season all seedlings were transplanted to a treeless section of the field close to where 'mycorrhizal' and 'non-mycorrhizal' soil had been collected. Two growing seasons later, the differences attributed to the original propagating treatments were still present. *Hebeloma* and *Lactarius* mycorrhizas continued to be relatively more abundant on roots of seedlings propagated in 'non-mycorrhizal' soil than on roots grown in 'mycorrhizal' soil. However, they were beginning to be superseded by *Inocybe* mycorrhizas which in turn were being replaced in 'mycorrhizal' soil by an increasing proportion of BCT mycorrhizas.

In addition to providing evidence of mycorrhizal succession - changes from *Hebeloma* to *Lactarius* and *Inocybe lanuginella* (Schroet.) Konrad and Maublanc in the 'non-mycorrhizal' soil and from *Inocybe* to BCT in the mycorrhizal soil - this experiment, described by FLEMING *et al.* (1984), demonstrates that the development of mycorrhizas during the years 3 - 4 after germination can be influenced by events during the first 8 weeks, a result of significance to the development of a rational approach to controlled inoculations.

B. HOST GENOTYPE X SOIL INTERACTIONS

While the experiment described above was being done, LAST *et al.* (1984) investigated the interactions between host genotype and soil. Axenically reared rooted cuttings of two clones (9.3D and 9.3G) of *Betula pubescens* Ehrh. were grown in unsterilised, groundwater gleys taken from two previously treeless locations (A & B). After 6 months in an unheated glasshouse, the cuttings were planted at the site from which soil B had been taken. Although the position of every fruitbody was recorded annually (LAST *et al.*, 1984) the results discussed here are restricted to those made 5 years after outplanting.

Irrespective of their origin cuttings propagated in soil B were associated, as they had been in earlier years with fruitbodies of *Hebeloma* spp. and *Inocybe petiginosa* (Fr.:Fr.) Gillet. In contrast in soil A, there is evidence of a strong plant x soil interaction. Whereas clone 9.3D was, as before, strongly associated with *Inocybe* clone 9.3G was associated with much larger numbers of fruitbodies of *Laccaria tortilis* ([Bolt.] S.F. Gray) Cooke, to the exclusion of *Hebeloma*.

This fruitbody evidence suggests that the range of early-stage fungi forming mycorrhizas on young trees is not only determined, as in previous section, by soil characteristics but also by host x soil interactions. In the one instance, clone 9.3D was associated with the same fungi irrespective of the

origin of the groundwater gleys being tested; in the other, the fungi associated with clone 9.3G differed considerably after propagation in the two soils.

Table 1. Percentage of sheathing mycorrhizas attributable to inoculant fungi at different times during the first 2 years growth of Betula pendula in a range of soils (LAST et al., 1985).

Inoculant fungi	Types of 'soil'			
	Sphagnum peat pH 3.4	Sedge peat pH 4.7	Mineral soils ex Farfield pH 5.4	Castlelaw pH 5.1
Hebeloma sacchariolens				
(a) Towards end of first season	25%	100%	100%	100%
(b) End of second season	Nil	100%	93%	99%
Paxillus involutus				
(a) Towards end of first season	100%	100%	100%	100%
(b) End of second season	44%	30%	19%	41%

C. INOCULATION X SOIL INTERACTIONS

To study the ability of early-stage fungi to colonise and form sheathing mycorrhizas with silver birch seedlings growing in a range of soils, seedlings previously inoculated during propagation with cultures of *Hebeloma sacchariolens* Quélet or *Paxillus involutus* (Batsch.:Fr.) Fr. were transplanted and grown in an unheated glasshouse for two seasons in four soils (two mineral soils and two peats) (LAST *et al.*, 1985).

At transplanting the inoculated seedlings, irrespective of treatment, had similar numbers of mycorrhizas, but thereafter differences started to appear. While all the mycorrhizas on *P. involutus*-inoculated seedlings were attributable to this fungus during the first season, they tended in the second season to be replaced in all 4 soils by an array of 'wild' type fungi. In fact, the mean proportion of mycorrhizas attributable to *P. involutus* decreased to 33% with a suggestion that the decrease was greater in one of the mineral soils than in the other 3 soils (Table 1). In contrast, the persistence of *H. sacchariolens* was very strongly related to soil type. From the end of the first season, inoculated seedlings growing in Sphagnum peat had few, if any, mycorrhizas attributable to *H. sacchariolens* while in the other soil types this fungus continued to dominate. Could the inability of *H. sacchariolens* to progress on seedlings growing in Sphagnum peat be related to extreme acidity? ALVAREZ *et al.* (1979) have shown that some mycorrhizal fungi are able to develop more prolifically on seedlings growing in mineral, than on those in organic soils, an observation of significance when considering the inoculation of seedlings to be outplanted to acidic forest soils common in the British uplands.

D. PERSISTENCE AND EFFECTS OF DIFFERENT SHEATHING MYCORRHIZAS ON THE GROWTH OF SITKA SPRUCE SEEDLINGS IN CONTRASTING SOILS

Sitka spruce (*Picea sitchensis* (Bong.) Carr.) seedlings grown from surface sterilised seeds were left uninoculated or inoculated during axenic propagation (8 wk) in vermiculite/peat permeated with either *Laccaria proxima* (Boud.) Pat.

or *Paxillus involutus*. They were then transferred to pots containing either peat or mineral soil, with different sets of replicates being destructively sampled at intervals.

Root systems, as judged by numbers of 'roots' per plant (see LAST *et al.*, 1985) were mostly larger in the brown earth than in the peat; they were also markedly larger, at the end of the second year than at the end of the first, the exception being the uninoculated control seedlings growing in peat. Inoculation with mycorrhizal fungi, consistently increased root system sizes. The absolute increases were independent of soil type in the first year but were much larger in the brown earth than in the peat in the second year. Despite the larger absolute increases in the brown earth at the end of the second year, inoculation had a relatively larger effect on seedlings growing in peat primarily because the uninoculated control seedlings were very small. Thus, inoculation increased root system sizes in the peat from 72 'roots' per plant to 660-960; in the brown earth from 880 to 2,000-2,500 (Table 2). In three of four instances, inoculation with *L. proxima* increased root system size more than inoculation with *P. involutus* but the differences were not statistically significant.

On the uninoculated series of seedlings, the proportion of 'roots' with mycorrhizas increased from 0.4% (yr 1) to 6% (yr 2) in peat, and from 24% (yr 1) to 50% (yr 2) in brown earth. As would be expected, inoculation increased the proportion of 'roots' with mycorrhizas, the relative effects being larger on seedlings growing in peat than on those in brown earth. In the first year, inoculation with *L. proxima* increased the proportion of 'roots' with mycorrhizas significantly more than inoculation with *P. involutus* but, by the end of the second year, this difference had lessened. However, at that time, there were major differences in the proportions of mycorrhizas that could be attributed to the different inoculant fungi. While all of the mycorrhizas on *L. proxima*-inoculated seedlings could be attributed to that fungus when growing in peat, only half could be when the seedlings were grown in brown earth. In contrast, only 2-8% of the mycorrhizas on *P. involutus*-inoculated plants could be attributed to this inoculant fungus. Despite this 'loss' of *P. involutus* during the second growing season, the benefits attributed to it during the first year were sustained. It seems, particularly in the peat, that the activities of inocula naturally occuring in that substrate had been stimulated. Thus, at the end of the second year, seedlings inoculated with *P. involutus* and growing in peat had 243 mycorrhizas per seedling attributable to naturally occurring inocula compared with 4 in the relevant uninoculated controls. In the brown earth series the comparable numbers are 1310 (inoculated) and 440 (uninoculated).

With the notable exception of the uninoculated series with very few mycorrhizas growing in peat, all seedlings grew significantly taller during the second season. Inoculation significantly and consistently increased heights: the magnitude of this effect was the same in the first year for seedlings growing in both soil types but, in the second, it was relatively larger for seedlings in peat than for those in brown earth. Interestingly, heights seem to be strongly correlated with sizes of root systems irrespective of the combinations of treatments. Thus, seedlings inoculated with *L. proxima* growing in peat developed roots and shoots of similar dimensions to those of uninoculated seedlings in brown earth.

CRITIQUE

Having previously established that fungi associated with trees of different ages have different abilities to colonize tree seedlings growing in unsterile

Table 2. Effects of inoculating Sitka spruce seedlings during propagation, with either Paxillus involutus or Laccaria proxima, on their subsequent performance when transplanted to contrasting types of soil.

	Assessments at end of year 1				Assessments at end of year 2			
	Inoculated during propagation with				Inoculated during propagation with			
	Uninoculated controls	*Paxillus involutus*	*Laccaria proxima*	L.S.D. p= 0.05	Uninoculated control	*Paxillus involutus*	*Laccaria proxima*	L.S.D. P=0.05
Height (mm)								
Peat	36.1	52.5	55.8	11.8	38.6	84.2	98.1	31.1
Brown earth	25.4	54.8	54.6		97.6	123.6	128.2	
Numbers of 'roots' per plant+								
Peat	47	110	110	(0.222)	72	660	960	(0.345)
	(1.679)	(2.040)	(2.057)		(1.860)	(2.821)	(2.984)	
Brown earth	75	110	170		880	2000	2500	
	(1.880)	(2.042)	(2.228)		(2.945)	(3.304)	(3.395)	
Proportion of 'roots' with mycorrhizas*								
Peat	0.4	26%	58%	(4.5)	6%	41%	67%	(15.1)
	(3.6)	(30.5)	(49.5)		(14.3)	(40.1)	(55.0)	
Brown earth	24%	35%	73%		50%	67%	63%	
	(29.3)	(36.0)	(58.6)		(44.9)	(55.1)	(52.6)	
Proportion of mycorrhizas attributable to inoculant fungus								
Peat	-	100%	100%		-	8%	100%	
Brown earth	-	69%	100%		-	2%	52%	

For statistical analyses: + Numbers (n) were transformed to log(n+1)
* Percentages were transformed to angles

conditions (DEACON *et al.*, 1983), the present series of results shows that the choice of fungus may, in the second instance, be influenced by soil type. It seems that *Laccaria proxima* is better able to continue forming mycorrhizas in peat soils than in mineral soils, whereas the opposite seems to be true for *H. sacchariolens*. Additionally, it has been found that events during a relatively short period of propagation, after germination, can greatly influence the types of mycorrhizas formed for a number of years thereafter. While this may be of intrinsic interest, the results of the experiments done to assess the effects of mycorrhizas on the growth of Sitka spruce seedlings suggest that numbers of mycorrhizas may be more important than the types of mycorrhizas (CLINE and REID, 1982) - a relationship that warrants further analysis.

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